

# Cy5.5-Arg-Arg-Arg-Arg-crosslinked iron oxide nanoparticle

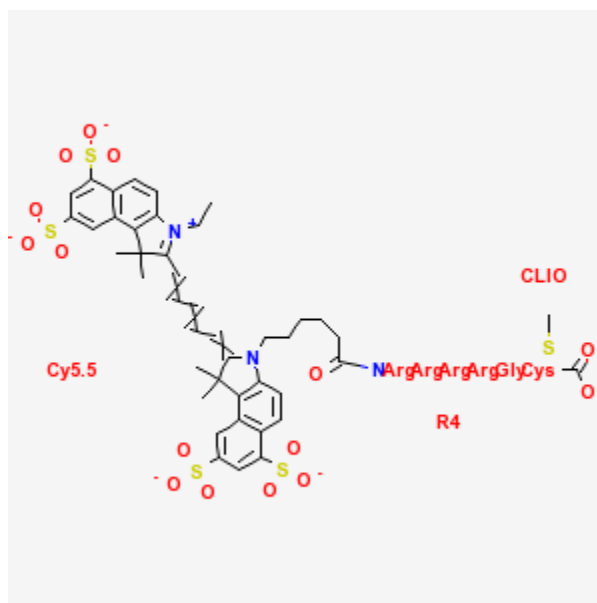
## Cy5.5-R4-SC-CLIO

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<b>Chemical name:</b>	Cy5.5-Arg-Arg-Arg-Arg-crosslinked iron oxide nanoparticle
<b>Abbreviated name:</b>	Cy5.5-R4-SC-CLIO
<b>Synonym:</b>	
<b>Backbone:</b>	Peptide
<b>Target:</b>	Proteases
<b>Mechanism:</b>	Proteolytic cleavage
<b>Method of detection:</b>	Optical and magnetic resonance
<b>Source of signal:</b>	Cy5.5 and iron oxides
<b>Activation:</b>	Yes
<b><i>In vitro</i> studies:</b>	Yes
<b>Rodent studies:</b>	Yes
<b>Other non-primate mammal studies:</b>	No
<b>Non-human primate studies:</b>	No
<b>Human studies:</b>	No



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## Background

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[PubMed]

Optical fluorescence imaging is increasingly used to obtain biological functions of specific targets *in vitro* and in small animals (1, 2). Near-infrared (NIR) fluorescence (700-900 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have wider dynamic range and minimal background because of reduced scattering compared with visible fluorescence detection. They also have high sensitivity resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a noninvasive alternative to radionuclide imaging *in vitro* and in small animals.

Magnetic resonance imaging (MRI) maps information about tissues spatially and functionally. Protons (hydrogen nuclei) are widely used to create images because of their abundance in water molecules. Water comprises >80% of most soft tissues. The contrast of proton MRI depends mainly on the density of nuclei (proton spins), the relaxation times of the nuclear magnetization (T1, longitudinal and T2, transverse), the magnetic environment of the tissues, and the blood flow to the tissues. However, insufficient contrast between normal and diseased tissues requires development of contrast agents. Most of the contrast agents affect the T1 and T2 relaxation of the surrounding nuclei, mainly the protons of water. T-2\* is the spin-spin relaxation time composed of variations from molecular interactions and intrinsic magnetic heterogeneities of tissues in the magnetic field (3).

Superparamagnetic iron oxide (SPIO) structure is composed of ferric iron (Fe<sup>3+</sup>) and ferrous iron (Fe<sup>2+</sup>). The iron oxide particles are coated with a protective layer of dextran or other polysaccharide (3). These particles have large combined magnetic moments or spins, which are randomly rotated in the absence of an applied magnetic field. SPIO is used mainly as a T2 contrast agent in MRI, although it can shorten both T1 and T2/T2\* relaxation processes. SPIO particle uptake into the reticuloendothelial system (RES) is by endocytosis or phagocytosis. SPIO particles are taken up by phagocytic cells, such as monocytes, macrophages, and oligodendroglial cells. A chimeric nanoparticles probe was constructed with a Cy5.5-R4 (L-Arg-Arg-Arg-Arg peptide) and cross-linked iron oxide (CLIO) nanoparticles to form Cy5.5-R4-SC-CLIO (4, 5). The Cy5.5-R4 part of the probe is activated by protease activity in the local environment, whereas the CLIO part provides spatial/anatomic information.

## Synthesis

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[PubMed]

Aminated CLIO consists of a core of SPIO, a cross-linked coating of dextran with amino groups, which was reacted with succinimidyl iodoacetate followed by R4 to produce R4-SC-CLIO (4, 5). Cy5.5 was attached to R4-SC-CLIO using *N*-hydroxysuccinimidyl (NHS) ester of Cy5.5 to yield Cy5.5-R4-SC-CLIO. Unreacted Cy5.5-NHS dye was removed using a PD-10 column. The sizes of the Cy5.5-R4-SC-CLIO nanoparticles were determined by laser light scattering to be 62 ± 7 nm. There were 15.5 R4 peptides and 1.79 Cy5.5 fluorophores per nanoparticle. The R1 and R2 relaxivities were 29.9 and 92.5 s<sup>-1</sup>mM<sup>-1</sup>, respectively.

## *In Vitro* Studies: Testing in Cells and Tissues

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[PubMed]

Incubation (for 200 min) of Cy5.5-R4-SC-CLIO (25 µg of Fe/ml) with cathepsin B (1.6 µg/ml) or trypsin (10 µg/ml) increased the NIR fluorescent intensity 27.4-fold by cleaving the L-Arg residues in the probe (4). Cy3.5-r4-SC-CLIO or Cy5.5-r4-SS-CLIO with D-Arg residues showed no increase in NIR fluorescence in the presence of the enzymes.

A mixture of Cy5.5-R4-SC-CLIO and Cy3.5-r4-SC-CLIO was incubated with different numbers of murine macrophages (5 X 10<sup>6</sup>, 10 X 10<sup>6</sup>, and 15 X 10<sup>6</sup> cells/0.1 ml) in the presence or absence of cathepsin inhibitors (5). The Cy5.5 and Cy3.5 fluorescence in the cells was measured after 6 h

of incubation. Increasing numbers of cells were associated with increases in fluorescence for both fluorophores, indicating that both probes were internalized by macrophages. Cy5.5 fluorescence increase was blocked by cathepsin inhibitors, while Cy3.5 fluorescence was not. This is because Cy5.5-R4-SC-CLIO was activated by macrophages, and Cy3.5-r4-SC-CLIO was not.

## Animal Studies

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### Rodents

[PubMed]

To study the feasibility of using Cy5.5-R4-SC-CLIO nanoparticles as a combined optical and MR contrast agent, mice received injections of 5.1 mg/kg (2 nmol) of Cy5.5-R4-SC-CLIO (4). After 18 h, MR and NIR fluorescence images were obtained from excised tissues. Uptake of Cy5.5-R4-SC-CLIO was evident in the spleen and lung from a loss of T2 signal as compared with a control mouse. There was good uptake in the liver, spleen, and kidneys, as measured by Cy5.5 signal. Whole-body imaging of a mouse that received an injection of Cy5.5-R4-SC-CLIO showed a loss of T2 signal from the axillary and brachial nodes by MR imaging. Reflectance imaging also showed Cy5.5 fluorescence signal from these nodes. These studies suggested a role of macrophages in the RES of the liver, spleen, and lung to accumulate the probe. Increased NIR fluorescence signal may be because of high concentrations of proteases in macrophages in these organs.

Mice received injections of a mixture of Cy5.5-R4-SC-CLIO and Cy3.5-r4-SC-CLIO, and selected tissues were excised 24 h after injection for NIR fluorescence measurement (5). The organ with the highest Cy5.5-to-Cy3.5 NIR fluorescence ratio was the liver (59.4), followed by the spleen (26.6), lung (25.5), kidneys (25.0), and muscle (17.0). The injection mixture had a ratio of 8.9. The increase in the ratio over the injection mixture indicates the activation of the Cy5.5 probe by cathepsin in these organs and not the activation of the Cy3.5-r4-SC-CLIO probe. Cy5.5-R4-SC-CLIO is capable of localizing its position through its magnetic properties (CLIO), providing functional information (protease activity) by optical imaging technology (Cy5.5-R4).

When CLIO is phagocytized, the exchangeable water is excluded; therefore, there is a loss of MRI signal. The NIR fluorescence signal increased because of hydrolysis by macrophage proteases in the nodes. Cy5.5-R4-SC-CLIO has a T1/T2 MRI signal in an aqueous environment and a low Cy5.5 fluorescence signal. When proteases cleave the R-R bond to form Cy5.5-RR and RR-SC-CLIO, NIR fluorescence is increased from Cy5.5-RR as compared with Cy5.5-R4-SC-CLIO, either in solution or inside the macrophages. The RR-SC-CLIO MRI signal is decreased if RR-SC-CLIO is formed inside the macrophages. The Cy5.5-R4-SC-CLIO MRI signal is also decreased if it is engulfed by the macrophages without hydrolysis, and in this case, little change in NIR fluorescence signal, as shown by protease inhibition.

### Other Non-Primate Mammals

[PubMed]

No publication is currently available.

## Non-Human Primates

[PubMed]

No publication is currently available

## Human Studies

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[PubMed]

No publication is currently available.

## References

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